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Some Aspects of the Symbionts of Cassiopeia frondosa

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BIOLOGY

Some Aspects of the Symbionts of Cassiopeia frondosa

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ABSTRACT — *Symbiodinium microadriaticum* were found localized in the mesoglea of *Cassiopeia*, the upside-down jellyfish. Axenic cultures of the symbiont were produced, utilizing chemically-defined media. Studies of the life cycle established the presence of isogametes which had previously been postulated. Reactions to various bands of the spectrum were noted, and tolerance tests for survival in darkness and under temperature stress were carried out. Nutritional experiments established nitrates as the best nitrogen source and verified the fact that vitamins B_{12} and Thiamine, though not essential for life, have a stimulating effect on the growth and motility of these zooxanthellae.

Symbiodinium microadriaticum lives as a symbiont in the upside-down jellyfish, *Cassiopeia,* and in some species of coral. Previous contributions regarding this organism have been largely the work of P. A. Zahl (with the National Geographic Society), J. J. A. McLaughlin (Fordham University and Haskins Labs, New York) and H. D. Freudenthal (Museum of Natural History, New York). Present research was done at the Haskins Labs, New York, under a grant from the National Institutes of Health.

Symbiodinium microadriaticum belongs to the zooxanthellae, having a golden-brown color and being classified with the dinoflagellates. Previous workers had been able to extract the symbiont from the host tissue, and had grown the organism in artificial media. They also had determined the major phases of the life cycle and the fact that the symbionts are nutritional autotrophs.

The present work began with the attempt to establish axenic cultures in a chemically-defined medium. *Cassiopeia* specimens were obtained from Florida. Symbionts were extracted by placing portions of the jellyfish (well covered with sea water) into a Waring blender and spinning for 5-7 minutes. The homogenate was centrifuged at medium speed. The supernatant was discarded, more seat water was added and again centrifuged. After 3 washings, most of the extraneous material had been removed, and the remainder was used as inoculum.

Five cc. of this material was inoculated into 10 cc. of a culture medium. It was found that ASP-8A, ASP II NTA, and ASP-VIII all gave ample support for good growth. (These basic dinoflagellate media were developed by L. Provasoli and J. J. A. McLaughlin, 1957, and modified by McLaughlin and Zahl, 1957). Basically, they consist of NaCI, KCI, K_2HPO_4, $MgSO_4$, $NaNO_3$, P II metal mix, traces of Vitamin B_{12} and Vitamin 8 A mix, with TRIS (hydroxymethyl aminomethane) as a buffer.

Cassiopeia, the host animal, harbors *Symbiodinium microadriaticum* as a symbiont in the mesoglea of the tentacles and around the rim of the umbrella. (Figure 1). The jellyfish thrives off the coast of Florida in comparatively shallow waters. (Figure 2)

The symbionts can be readily extracted, as indicated above, and grown in axenic cultures. The cells are spherical, measuring from 7-9 micron in diameter, have an in-

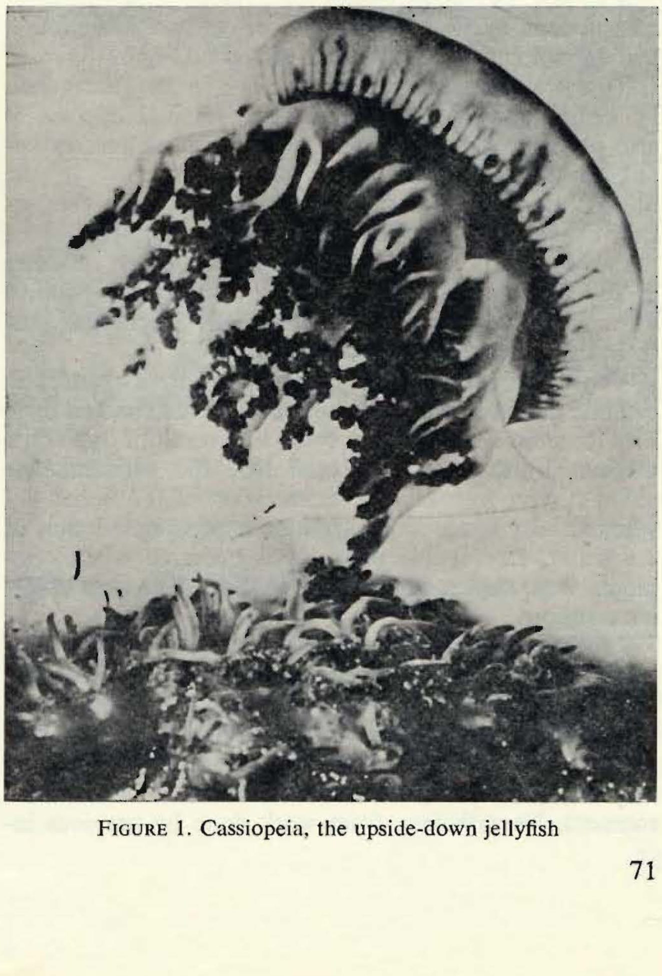

FIGURE 1. Cassiopeia, the upside-down jellyfish

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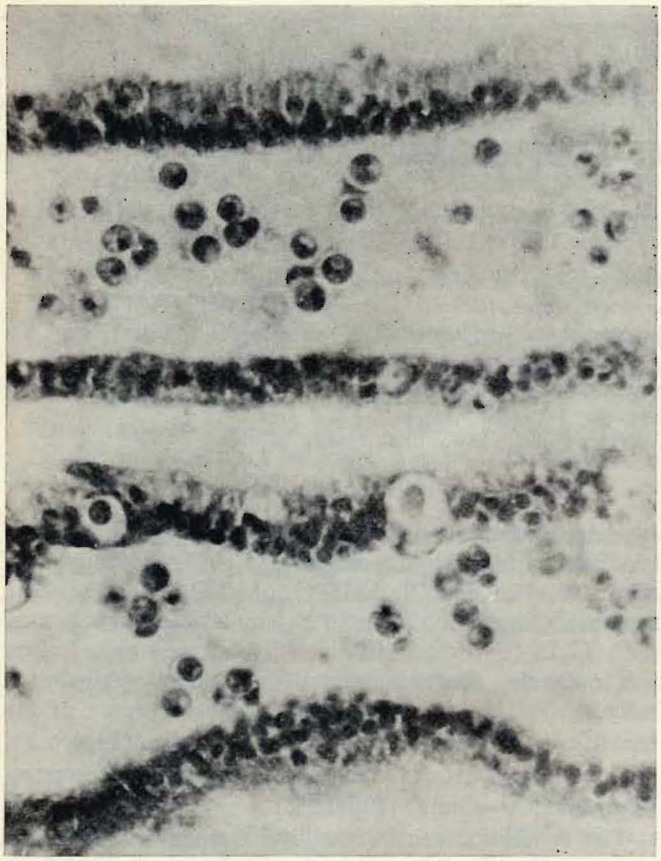

FIGURE 2. Section through the tentacle of Cassiopeia showing symbionts in the mesoglea. X 1000

conspicuous nucleus and an amber-colored assimilation body containing accumulations of food. (Figure 3).

This organism can survive through many generations by simple fission of the vegetative stage. However, it also produces zoosporangium which produce gymnodinioid zoospores, aplanospores, and autospores. In the present research, the author has established the production of isogametes which are less than half the size of the zoospores. These presumably effect a union resulting in the development of the usual, dominant vegetative form. Giant cells appeared occasionally in cultures, but their significance was not determined.

Vegetative cells showed great tolerance to changes in osmotic pressure. However, cells that were extracted from host tissue in distilled water lysed as a result of hypotonic tension. Further work showed that the zooxanthellae could survive in a saline concentration of 0.3% but developed best when concentrations approximated that of sea water. Irreversible plasmolysis occurred when symbionts were subjected to 8.0% to 10.0% sodium chloride solutions.

In vitro cultures seem to indicate that development of motile forms can be associated with factors other than light. Large numbers of gymnodinioid forms were found in cultures having ample supplies of nutrients, especially nitrates, phosphates, and vitamins. Zoospore production may be enhanced by various circumstances of the environment. Nevertheless, from work done by previous investigators (McLaughlin and Zahl, 1966), the formation of zoospores is not obligatory for survival, and symbionts are capable of survival for many generations by vegetative methods alone, at least when cultured *in vitro*.

Temperature Tolerance

Symbionts grow best in an air-conditioned room kept at a temperature of 21-26° C. Attempts were made to determine upper and lower levels of temperature tolerance. For this purpose organisms were cultured in Ionager #3 and exposed to temperatures ranging from $-15°$ C to 50° C. There was no survival at either extreme. Growth was poor at 28° C, and death ensued upon exposure to 33° C for as long as 24 hours.

Light Preference

Moderate illumination was supplied by vertical banks of eight 40-watt, cool, white, natural fluorescent lamps. Cultures were placed at 15-20 cm. from light banks and rotated occasionally to secure uniform exposure to the illumination. Average light intensity was 185-200 foot-candles. Lights were turned off in the evening and turned on in the morning to simulate the day-night cycle of the host organism.

In an attempt to determine preference for various bands of the spectrum, fiters (red, yellow, green, blue and violet colors) were used to screen out the respective wave lengths. Lack of good growth response in cases where the intermediate bands were screened out, and good growth at both extremes, may be related to pigmen-

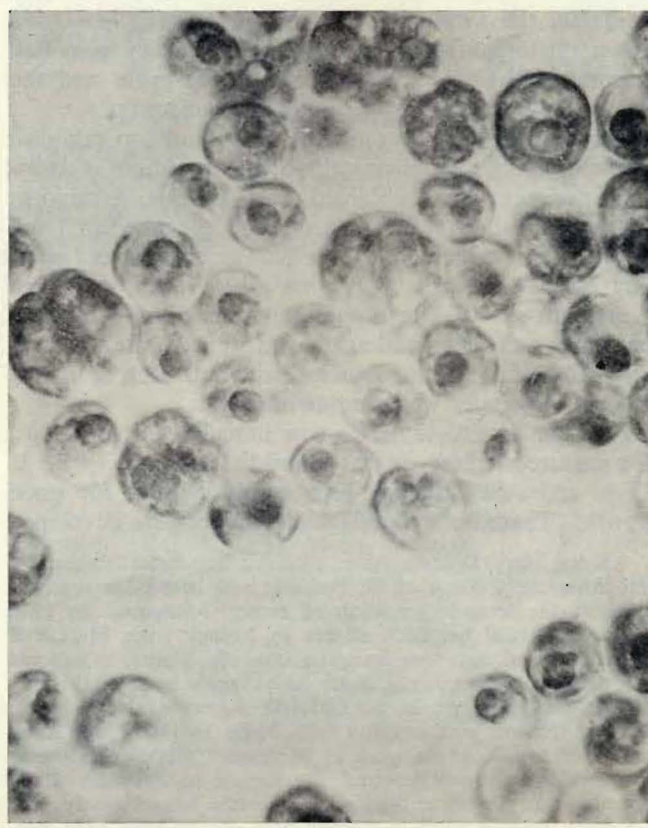

FIGURE 3. Symbionts of *Cassiopeia* after extraction from host tissues and cultured in chemically defined medium.

tation of the symbiont cells. Analysis of pigments of these organisms indicate the presence of chlorophylls, xanthophylls, carotenes and perhaps others (McLaughlin, Zahl, Nowak and Marchisetto, 1963).

Survival in Darkness

Some cells survived for as long as 16 weeks in total darkness. Presumably the organisms assumed a state of quiescence, since no observable motility or cell proliferation occurred after the ninth week. Some bleaching was noted after a period of 4 weeks in darkness. Upon restoration to normal conditions of lighting, a lag period ensued before the usual cell activities were resumed.

Nitrogen Sources

Nitrates of sodium and ammonia at concentrations of 5.0-20 mg % gave best responses of both growth and motility. Nitrites, urea, and uric acid also proved to be utilizable as nitrogen sources, but growth was less vigorous than with nitrates, and the latter two proved toxic at comparatively low concentrations.

Phosphates

Sodium glycerolphosphate supplied adequate amounts of phosphate for good symbiont growth at concentrations of 1.0 mg % to 7.0 mg %. Above and below this range, development was hampered. High phosphate levels stimulated bacterial proliferation in non-axenic cultures.

Vitamins

Studies made on cultures supplied with B_{12} in concentrations of .1 millimicron % to 25 millimicron %, indicated a stimulating effect on growth and production of the dinoflagellate phase. Thiamine when supplied in comparable amounts appeared to stimulate dinoflagellate production, but not vegetative growth. The organisms, however, do not show an absolute vitamin requirement.

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Increased State Placements

Records of the Institute of Technology at the University of Minnesota show that 10 years ago, in 1958, thirty percent of the Institute of Technology graduates obtained employment in Minnesota. In 1968 sixty percent of the I. T. graduates were employed in the state.